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# OBSERVATIONS ON THE RESPIRATORY ENZYMES OF VARIOUS LIFE-STAGES OF CHIRONOMUS PLUMOSUS, CHIRONOMUS STAEGERI, AND AEDES AEGYPTI[1]

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The pattern of respiratory enzymes in the midge genus *Chironomus* (*Tendipes*) is of interest for several reasons.[2] Most of the insect tissues investigated by Zebe and McShan (1957) exhibited very little activity of lactic dehydrogenase, an enzyme essential to the operation of anaerobic glycolysis in vertebrate tissue. Yet the larvae of some species of *Chironomus* regularly survive weeks or months in oxygen-free surroundings at the bottoms of eutrophic lakes. Furthermore, the various stages in these insects' life-histories differ markedly in their requirements for energy and in their tolerance of anoxia.

This report describes some measurements of the activity of the anaerobic glycolytic system in larvae of two species of the genus *Chironomus*, and of the succinoxidase and cytochrome oxidase systems in larvae, pupae, and adults of these species and of the mosquito, *Aedes aegypti*.

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## Materials and Methods

### *A. Animal material*

The larvae of *C. plumosus* and of *C. staegeri* are found in great abundance burrowing in the bottom sediments of stratified eutrophic lakes where they are frequently subjected to several weeks or more of oxygen deprivation in late summer and fall. In the laboratory, larvae of *C. plumosus* are more resistant to anoxia than those of *C. staegeri* (Neess and Della Croce, unpublished data).

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[2] For a discussion of the use of the name *Chironomus* the reader is referred to Neess and Dugdale (1959).

After a period of larval development, lasting up to two years for *C. plumosus* and for an unknown but probably shorter time for *C. staegeri,* the first indications of imminent pupation and emergence appear. At this time, the animals are marked by a darker coloration of the anterior segments and by the appearance of black pigment at the site of the adult eyes. Larvae which show no external changes preparatory to pupation will be referred to hereafter as "normal" larvae, while those in the stage just described will be designated as "pre-pre-pupational." This stage, which may last for several weeks, is followed immediately before pupation by the "pre-pupational," in which the thoracic segments become markedly swollen at the site of the future wing muscles and take on a distinctly lighter color than the rest of the animal. Often groups of pre-pupational larvae exhibit a bimodal weight distribution which Dugdale (1956) has shown to be correlated with sex, the heavier animals representing the future females.

These later larval stages are somewhat more sensitive to oxygen lack than are the earlier stages. They also seem to be more active since they are sometimes taken, apparently while swimming towards the surface, in plankton nets. In both traits, they foreshadow the pupae, which are completely intolerant of oxygen lack and which swim actively towards the surface in preparation for emergence.

The final transformation of *Chironomus* pupae into adults takes place at the surface of the lake, usually at dawn or dusk. The freshly emerged adults rest on the surface of the water briefly and then fly towards shore, where they mate during the day. The males are more active in this process, forming large swarms whose members hover rapidly for extended periods. The females rest on the ground or on leaves most of the time, but occasionally one flies through the swarm, copulates in flight, and returns to rest. At dusk the females fly back onto the lake and lay their eggs in compact sticky masses on its surface. Females which have not laid their eggs are here designated as pre-ovulatory. Those that have laid their eggs are designated as post-ovulatory. The subsequent fate of the females is not known, though the results of this investigation may shed some light on the question. The adults do not live for more than several days after emerging from the lake.

*Aedes aegypti* also undergoes a transformation from a less active larva through a more active pupa to a highly active adult, but it does not change in its oxygen requirements, being an obligate aerobe at all stages.

*Aedes* were reared from eggs kindly provided by the U. S. Public Health Service Laboratories of the Communicable Disease Center at Savannah, Georgia. Larvae and pupae of *Chironomus* were obtained by dredging bottom mud from Lake Mendota near Madison, Wisconsin, with a modified Ekman dredge (Welch, 1948, p. 176) and washing it through a wire-mesh screen with a spray of water. Adult *Chironomus* were obtained immediately after their emergence and while the females were laying eggs, by attracting them to a lantern on a boat. Swarming males and females on the ground were captured by netting. All adults were used within six hours and all larvae and pupae within two days after they were collected.

### *B. Methods*

Enzyme activities were generally determined by Warburg manometry, using tissue homogenates. These were prepared by placing weighed live animals into

a sharp-pointed ground glass homogenizer, adding ice-cold distilled water, and grinding with a motor-driven pestle. All tissues were kept in ice after being homogenized. No more than fifteen and usually less than five minutes elapsed between the time the animals were killed and the beginning of the enzyme determination. Readings of pressure changes were made at ten-minute intervals, and the average of the first four or six readings was used. Two to four animals were homogenized together to reduce the effect of individual variations in activity. Larvae and pupae were blotted on filter paper before being weighed to remove the film of water which clings to their surfaces. Adult insects were chilled to prevent them from flying before being weighed and placed in the cup.

The activity of the glycolytic enzyme system was determined by the technique of Novikoff (1948), using a 95% nitrogen, 5% carbon dioxide atmosphere. After the incubation period, the contents of one-half of the Warburg flasks were deproteinized by the addition of equal volumes of 10% perchloric acid and centrifuging, and the amounts of lactic acid formed in these flasks were then determined by the method of Barker and Summerson (1941).

Succinoxidase activities were measured by the method of Schneider and Potter (1943). In some cases, cytochrome *c* or the substrate, sodium succinate, was omitted. Cytochrome oxidase was studied principally by the method of Schneider and Potter (1943), using sodium ascorbate as the substrate. Corrections were made for the autoxidation of the ascorbate by using different amounts of tissue in the individual flasks of a series, and calculating the autoxidation by extrapolating the total oxidation to a tissue concentration of zero. A few determinations of cytochrome oxidase activity were made by the microspectrophotometric method of Cooperstein and Lazarow (1951). All chemicals used were reagent grade commercial preparations.

All the determinations of glycolytic and cytochrome oxidase activities and of the succinoxidase activities of *C. staegeri* and of *A. aegypti* were made at 38° C. Two series of measurements of the succinoxidase activity of *C. plumosus* were made. The first, which included observations on a greater number and variety of adults than the second, was made at 15° C., approximately the highest temperature which the larvae experience in their natural habitat. The second, at which all stages exhibited greater activity, was made at 38° C.

All results are expressed in terms of gas exchange or lactate production per unit wet weight of tissue. Those dry weights which were determined represented a quite constant proportion of the animals' wet weights, ranging from 13% for *C. plumosus* larvae to 26% for *C. plumosus* adults. The final concentrations of tissue in the flasks were between 0.3 and 20.0 mg. wet weight per ml. The concentrations of the different tissues used were chosen so that the changes in pressure would be of a conveniently measured magnitude.

In some cases, the concentration of tissue within the flasks was varied among replicated determinations in order to observe the effects of such variations on the activity. Succinoxidase activity proved to be related to tissue concentration in an almost linear manner in all cases in which the effect was measured. Variations in the tissue concentrations in the determinations of cytochrome oxidase were used in calculating the substrate oxidation rate, and so an assumption of linear response to such variation is necessary. In all cases, variations in unit activity caused by

variations in concentration would contribute to the standard error which was calculated for the mean activity of each tissue. Therefore, it is reasonable to assume that a small standard error or a statistically significant difference between the means of two tissue types indicates that differences in concentration did not affect unit activities significantly.

## Results and Discussion

### *A. Glycolysis*

The formation of lactate and the evolution of $CO_2$ by the larval tissue homogenates in the first series of experiments indicated the presence of a glycolytic system. The omission of both hexose diphosphate and glucose, *i.e.*, of all carbohydrate substrates, reduced the rate of lactate production by 90% while the carbon dioxide production was reduced by only 45%. This indicates that a source of hydrogen ions other than lactic acid was present in the reaction, since any reduction in pH would cause the release of $CO_2$ from the bicarbonate in the flasks. Since the evolution of $CO_2$ was prevented only by the omission of ATP from the vessels it is probable that the larvae, like the cockroach and grasshopper muscle studied, respectively, by Barron and Tahmisian (1948) and Humphrey and Siggins (1949) contain an ATP-ase capable of removing an acidic phosphate group from this nucleotide. Therefore, conclusions about the glycolytic capabilities of the larvae have been based on lactate production rather than on the evolution of $CO_2$.

One indication was found of a qualitative difference between the larval enzyme pattern and those found by Novikoff (1948) in tumors and Utter *et al.* (1945a, 1945b) in mammalian nervous system tissues. In the latter cases the addition of phosphorylated hexose was indispensable for the operation of the system. The rate of production of lactate of three samples of larval tissues, incubated without added HDP, was lowered by only about 40%.

### *B. Succinoxidase*

Table I shows the means and standard errors for oxygen uptake of the various preparations. The endogenous oxidation of homogenates of samples of all the life stages of *C. plumosus* was quite low at 15°, and there were no differences among them. The effect of the omission of cytochrome *c* from the reaction mixtures was less in the less active tissues, *e.g.*, those from larvae and pre-ovulatory females (15°), while in the more active tissues the oxygen uptake was reduced by the same proportion as that found by Potter (1941) and by Schneider and Potter (1943) in rat liver under the same conditions.

At 15°, the succinoxidase activity of homogenates of larvae of *C. plumosus* was little greater than their endogenous oxidation. The pupae showed a highly significant increase in activity over the larvae and a highly significant difference between the sexes, the male pupae having an oxygen uptake over twice that of the female. The rate of oxygen uptake of freshly emerged adult males was over twice that shown by the male pupae, and that of actively swarming males was slightly higher yet. However, the activities of freshly emerged female adults showed no similar increases, but rather fell to levels approaching those of larvae.

Harvey and Beck (1953) and McShan, Kramer and Schlegel (1954) had also found differences in succinoxidase activity between the sexes in an insect, the cockroach *Periplaneta americana*, and Barron and Tahmisian (1948) found a sexual difference in the over-all oxygen uptake of *Periplaneta* thoracic muscle. In these instances the ratio of oxidative activity in the male to that in the female was only two or three, whereas adult male chironomids displayed a rate ten times that of the females. At first it was thought that this difference was correlated with the fact that the difference between the activities of the sexes is greater in *Chironomus* than in *Periplaneta*. This explanation was shown to be incorrect when homog-

TABLE I

*Succinoxidase activity in cm.³/gm. wet weight/hr.*

| Life stage | *C. plumosus*, 15° | *C. plumosus*, 38° | *C. staegeri*, 38° | *A. aegypti*, 38° |
|---|---|---|---|---|
| Larvae | | | | |
| "Normal" | .43 ±.05 | 1.59 ±.06 | 2.92 ±.10 | .72 ±.01 (2 days after hatching) |
| | .13* | .45** | 2.66 ±.12 (sw.) | 1.51 ±.22 (5 days after hatching) |
| | .41 ±.15** | | | 2.35 ±.07 (6–13 days after hatching) |
| | | | | .68 ±.15 (6–13 days after hatching)* |
| | | | | .91 ±.51 (6–13 days after hatching)** |
| Pre-pre-pupational, ♂ | | 1.84 ±.08 | | |
| Pre-pre-pupational, ♀ | | 1.89 ±.08 | | |
| Pre-pupational, ♂ | | 2.24 ±.10 | 3.60 ±.04 | |
| | | 0* | | |
| Pre-pupational, ♀ | | 1.94 ±.10 | 3.32. ±11 | |
| | | 0* | | |
| Pupae, ♂ | 2.54 ±.18 | 12.26 ±.28 | 9.22 ±1.15 | 2.79 ±.09 (♂ + ♀) |
| | .12* | | | .91 ±.04* (♂ + ♀) |
| | 1.09** | | | .65 ±.04** (♂ + ♀) |
| Pupae, ♀ | 1.13 ±.04 | 8.24 ±.34 | 7.21 ±.28 | |
| Adult, ♂ | | | | |
| Freshly emerged | 5.85 ±.32 | | | 15.29 ±.61 |
| | .15 ±.02* | | | |
| | 2.35 ±.33** | | | |
| | 11.7 ±.23† | | | |
| Swarming | 7.65 ±.93 | | | |
| | 4.20 ±.37** | | | |
| With eggs | .42 ±.03 | | | |
| Adult, ♀ | | | | 14.82 ±.17 |
| Freshly emerged | .59 ±.23 | | | 12.04 ±.41 (after blood meal) |
| | .18** | | | |
| | 6.91 ±.80† | | | |
| Pre-ovulatory | .46 ±.05 | 2.08 ±.66 | | |
| | .45 ±.06** | 16.70 ±3.3† | | |
| Post-ovulatory | | | | |
| Lake caught | 1.43 ±.40 | | | |
| Land caught | 3.79 ±.26 | | | |
| | 2.43 ±.46** | | | |

* =no substrate; ** =no cytochrome; † =heads and thoraces; (sw.) =swimming.

enates of females which had laid their eggs were found to have a succinoxidase activity some seven times higher than homogenates containing unlaid eggs.

The hypothesis that the eggs of *C. plumosus* were capable of inhibiting the activity of the succinoxidase enzyme system was supported by the fact that homogenates of thoraces, alone, of pre-ovulatory females displayed a succinoxidase activity of the same order of magnitude as homogenates of the entire post-ovulatory animals, which suggested that there was no change in the enzyme system itself at the time the eggs were laid. The inhibitory effect of the eggs was directly demonstrated by homogenizing an egg mass, collected immediately after it had been laid, together with adult male midges in a ratio of two parts by weight of egg mass to five parts of whole male insect. This was approximately the ratio of the weight of a

gravid abdomen to the weight of an adult female. The homogenate of male adult with eggs had an activity as low as that of the entire pre-ovulatory females, while a control without eggs showed a typically high activity. Since the egg masses made up only 40% of the weight of tissue present, while their presence reduced the succinoxidase activity by about 90%, it seems probable that they were actively inhibiting the enzyme system and not merely diluting it with inert tissue.

As Table II indicates, the developed egg masses, when homogenized with rat liver in the same proportion as with male *Chironomus,* reduced its activity by 70%, both at 15° and at 38°, but the egg-containing abdomens of the females, similarly homogenized, had no such effect. This difference may well be related to changes in the chemistry of the egg masses following fertilization or oviposition. The difference between the response to the undeveloped eggs by the tissues of the females and by the rat liver, and the greater magnitude of the effect of the eggs on the adult males may indicate a specific difference in the properties of the enzymes of the two forms. It remains to be seen whether the inhibiting effect of the egg masses has any functional significance for the insects.

TABLE II

*Inhibition of mammalian succinoxidase; activity expressed as cm.³ $O_2$/gm. wet weight/hr.*

| Tissue and temperature | Control | With *C. plumosus* egg-laden abdomen | With *C. plumosus* egg masses |
|---|---|---|---|
| Rat liver 15° | 4.06 | 4.02 ± .27 | 1.09 ± .52 |
| Rat liver 38° | 25.68 ± 1.34 | 21.04 ± 1.26 | 4.89 ± .65 |
| | | With *A. aegypti* egg-laden abdomen | With *A. aegypti* eggs |
| Mouse liver 38° | 32.49 ± 1.39 | 41.05 ± .05 | 30.34 ± .38 |

Female midges captured on the shore were readily identifiable as pre-ovulatory or post-ovulatory by the shape of their abdomens, those of the former being swollen and round in cross-section while those of the latter were flat and narrow. The succinoxidase activity of all the members of the former group was distinctively low and that of the latter high. The condition of the midges captured on the lake was more ambiguous. A number of these animals were classified as post-ovulatory whose abdomens were not as completely reduced as those of the animals on shore or which were taken immediately after they were seen to lay eggs. The succinoxidase activity of this group of "post-ovulatory" females was significantly lower than that of the animals taken on land and it showed greater variability. These results seem to indicate that the female midge lays a number of egg masses in separate places on the lake and then returns to shore.

The elimination of the inhibition by the egg masses did not entirely destroy the difference between the sexes, but it did reduce it to the same order as that found in other insects. The rate of oxygen uptake of homogenates of entire swarming males was twice that of the post-ovulatory females. The same ratio was found when the activities of only the heads and thoraces were compared. The absolute

values of the latter were 50–80% higher than those of the entire animals. These results might be expected in view of the very high metabolic rates generally displayed by the thoracic flight muscles of insects. Another indication that the muscles of insects may vary in the level of enzymes of the Krebs cycle was reported by Brooks (1957), who found differences between the succinoxidase activities of different muscles of individuals of the American cockroach as well as between those of the sexes.

At 38°, the activity of the larval succinoxidase was four times that at 15°, indicating a $Q_{10}$ for the system of about two. There was no endogenous oxidation at the higher temperature. Pre-pre-pupational larvae at 38° showed a significantly higher activity than "normal" larvae. There was no difference between the mean activities of the heaviest and lightest individuals of this group, though, as suggested above, they very probably represented female and male. The mean activities of pre-pupational larvae were higher still, and a small, though not significant, difference between the sexes was found. As at 15°, the male pupae showed a sevenfold increase in activity over the larvae, and the female pupae a somewhat smaller increase. The activities of homogenates of whole pre-ovulatory adult females were again little more than those of the larvae, while those of homogenates of thoraces alone were eight times as high.

Tissues from pre-pupational larvae of *C. staegeri*, like those of *C. plumosus*, were somewhat more active than those from "normal" ones, and those from the males were more active than those from the females. The pupae were more active still, but the difference between the activities of pupae and of pre-pupational larvae was less than in *C. plumosus*. The difference in activity between male and female pupae was not significant.

The rate of oxygen uptake by the preparations of *staegeri* larvae was almost twice that shown by preparations of *plumosus* larvae, but the activity of *staegeri* pupae was less than that of *plumosus* pupae. One group of *staegeri* larvae which were taken in a plankton net showed a lower than average rate of succinoxidase activity, perhaps indicating that swimming behavior begins earlier in this form than in *plumosus*.

Since the *Aedes* used in this work were reared in the laboratory from the egg, the development of their enzyme activities could be followed from an earlier stage than that of *Chironomus*. Larvae examined two days after hatching showed a low level of activity, scarcely higher than that of the endogenous oxidation. After five more days of development at a temperature of ca. 20°, the activity had doubled and after eight days, had levelled off at a rate some three times higher than the original one. This final rate was between those shown by larvae of *C. plumosus* and *C. staegeri*.

The omission of cytochrome *c* from homogenates of either larvae or pupae reduced the activity to a level barely above that of the endogenous respiration. The latter was four times higher at a temperature of 38° than the endogenous respiration of *C. plumosus* larvae at 15°.

The pupae of *A. aegypti*, which were not differentiated by sex, had a succinoxidase activity only slightly higher than that of the larvae. The activity of the adults was some six times as high as that of the larvae or pupae and showed no sexual difference.

After a blood meal, the activity per unit weight of the females declined slightly but this may be due to the lower activity of the ingested blood which made up a substantial part of the weight of the insect. Two attempts were made to measure any possible inhibition of succinoxidase by *Aedes* eggs. In the first, mouse liver was homogenized together with dry and presumably viable eggs. In the second, mouse liver was homogenized together with the abdomens of female mosquitoes which had received a blood meal. In neither case was the succinoxidase activity of the mouse liver treated with eggs or abdomens reduced below that of untreated homogenates.

### *C. Cytochrome oxidase*

Table III shows the cytochrome oxidase activity of the various homogenates in cm.$^3$ oxygen taken up per gram wet weight per hour. These activities are in all cases greater than the succinoxidase activities of the same tissues, the ratio between

TABLE III

*Cytochrome oxidase activity at 38° in cm.$^3$/gm. wet weight/hr.*

| Life stage | *C. plumosus* | *C. staegeri* | *A. aegypti* |
|---|---|---|---|
| Larvae | | | |
| "Normal" | | | 14.97 ± 1.68 |
| 10 meters and deeper | 7.70 ± .38 | 21.48 ± .61 | |
| | 1.99 ± .35** | | |
| | 0* | | |
| 9 meters and shallower | 13.03 ± .42 | | |
| Pre-pupational, ♂ | 15.89 ± 2.62 | | |
| Pre-pupational, ♀ | 7.13 ± 2.43 | | |
| Pupae, ♂ | 37.90 ± .60 | 57.07 ± 9.28 | 25.86 ± .06 (♂ + ♀) |
| Pupae, ♀ | 35.75 ± .75 | 42.16 ± 6.56 | |
| Adult, thorax, ♂ | 119.70 ± 18.50 | | |

* = no substrate; ** = no cytochrome.

the two varying between three and ten. The changes in activity of cytochrome oxidase during development follow much the same pattern as do those in succinoxidase activity, but there is no evidence for an inhibition of cytochrome oxidase by the developing eggs of *C. plumosus*. The greater individual variations made some of the results less clear-cut.

The "normal" larvae of *C. plumosus* fell into two distinct groups. The majority showed an oxygen uptake of 7.7 ± 0.4 cm.$^3$/g./hr. One group, taken in late September from a relatively shallow area of Lake Mendota in which higher temperatures and oxygen concentrations are found during summer than in the habitat of the first group, had an uptake of 13.0 ± 0.4 cm.$^3$. These larvae showed no external sign of imminent pupation, though animals from this area of the lake generally pupate earlier than those deprived of oxygen earlier in the season. This may indicate either that previous oxygen deprivation reduces the activity of larval cytochrome oxidase or else that changes in cellular respiration preparatory to pupation may precede visible morphological changes.

Male pre-pupational larvae showed a slightly higher cytochrome oxidase activity than the "normal" group from shallow water; females showed a lower, though not significantly lower, activity. As with succinoxidase, the cytochrome oxidase activity of the pupae was some five times higher than that of the larvae and the activity of the male adults was three times that of the male pupae. There was no significant difference between the activities of male and female pupae.

*C. staegeri* and *A. aegypti* larvae displayed, respectively, three and two times the cytochrome oxidase activity of *C. plumosus* larvae, but the *staegeri* pupae showed only 1.3 times the activity of *plumosus* pupae, and the activity of *Aedes* pupae was less than that of *plumosus* pupae. It is of some interest that both the increases in activity of the two oxidative enzyme systems studied and the reduction of tolerance to anoxia which accompany pupation are greatest in *C. plumosus,* intermediate in *C. staegeri,* and least in *A. aegypti.*

## Additional Observations

A small number of observations on the oxidation of reduced cytochrome *c* by homogenates of *C. plumosus* and *C. staegeri* larvae were made using the microspectrophotometric method of Cooperstein and Lazarow (1951). These measurements were made at room temperature, *i.e.*, ca. 25° C. *C. staegeri* was found to have the more active cytochrome oxidase by this method as well, but the difference between the two species was not so great as when measured manometrically.

The effect of cyanide on the total oxidative metabolism of some of the subjects was studied in two ways. The oxygen uptake of intact larvae of *C. staegeri* and *A. aegypti,* maintained without food in a synthetic lake water medium, was measured by Warburg manometry at 20° C. and the effect of concentrations of sodium cyanide varying from $10^{-2}$ to $10^{-5}$ *M* was noted. The effect of $10^{-4}$ and $10^{-5}$ *M* cyanide on the oxidation of reduced cytochrome *c* by homogenates of larvae of *Chironomus* was also observed.

The addition of $10^{-4}$ *M* NaCN to their medium reduced the oxygen uptake of live larvae of both species by 30–50%, and higher concentrations caused proportionally greater reductions. Cyanide at $10^{-4}$ *M* concentration completely blocked the oxidation of cytochrome, and $10^{-5}$ *M* cyanide reduced it by 70%.

The ability of *C. plumosus* larvae to maintain a normal level of oxygen uptake in the presence of low external concentrations of oxygen, and the extremely low oxygen tensions at which the hemoglobin dissolved in their hemolymph unloads oxygen (at .00079 atmospheres, or 0.6 mm. Hg, according to Fox, 1945) has led to the suggestion (Prosser, 1952, p. 323) that the oxidative enzyme pattern of the larvae may be modified to permit the transfer of oxygen at very low concentrations.

To test this suggestion, two Warburg measurements of cytochrome oxidase were conducted under an atmosphere of 1% oxygen and 99% nitrogen. Under these conditions, no oxygen was taken up, indicating that if an enzyme modification such as that suggested exists, it probably involves a circumvention of cytochrome oxidase rather than an increase in its efficiency or affinity for oxygen. This possibility is also suggested by the fact that some insects are resistant to strong concentrations of cyanide. For example, the strongly aerobic larvae of caddis flies have been observed in this laboratory to survive in $10^{-2}$ *M* NaCN for more than twenty-four hours. Furthermore, Schneiderman and Williams (1954) have di-

rectly demonstrated a cytochrome oxidase by-pass in the diapausing silkworm pupa. Since much of the respiration of the *Chironomus* larvae studied here was eliminated by treatment with cyanide, it seems that the terminal oxidases of insects display considerable variety.

## Summary

1. Larvae of *Chironomus plumosus* and *Chironomus staegeri* have an active glycolytic enzyme system.

2. The succinoxidase and cytochrome oxidase systems of *C. plumosus, C. staegeri,* and *Aedes aegypti* are least active in the larval stage, more active in the pupal stage and most active in the adult stage. The increase in activity from larval to pupal stage is greater in those species, *C. plumosus* and *C. staegeri,* in which the larva, but not the pupa, is resistant to anoxia than in *A. aegypti,* in which neither is resistant.

3. Male pupae and adults of *C. plumosus* have a higher rate of succinoxidase activity than females. No sexual difference was found between the cytochrome oxidase activities of pupae or between the succinoxidase activities of *A. aegypti* adults.

4. The developing eggs of *C. plumosus* exert an inhibitory effect on the succinoxidase system of the insect and of rat liver.

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